

PNAS Classification: Biological Sciences, Evolution

Receding ice drove parallel expansions in Southern Ocean penguins.

Theresa L. Cole^{1,2,a}, Ludovic Dutoit^{1,b}, Nicolas Dussex^{3,4,b}, Tom Hart^{5,b}, Alana Alexander^{4,b}, Jane L. Younger⁶, Gemma V. Clucas^{7,8}, María José Frugone^{9,10}, Yves Cherel¹¹, Richard Cuthbert^{12,13}, Ursula Ellenberg^{14,15}, Steven R. Fiddaman¹⁶, Johanna Hiscock¹⁷, David Houston¹⁸, Pierre Jouventin¹⁹, Thomas Mattern¹, Gary Miller^{20,21}, Colin Miskelly²², Paul Nolan²³, Michael J. Polito²⁴, Petra Quillfeldt²⁵, Peter G. Ryan²⁶, Adrian Smith¹⁶, Alan J. D. Tennyson²², David Thompson²⁷, Barbara Wienecke²⁸, Juliana A. Vianna¹⁰, Jonathan M. Waters¹

¹ Department of Zoology, University of Otago, PO Box 56, Dunedin 9054, New Zealand.

² Manaaki Whenua Landcare Research, PO Box 69040, Lincoln, Canterbury 7640, New Zealand.

³ Department of Bioinformatics and Genetics, Swedish Museum of Natural History, Box 50007, Stockholm, Sweden.

⁴ Department of Anatomy, University of Otago, PO Box 56, Dunedin 9054, New Zealand.

⁵ Department of Zoology, University of Oxford, 11a Mansfield Road, South Parks Road, OX1 3SZ, UK.

⁶ Milner Centre for Evolution, University of Bath, Claverton Down, Bath, BA2 7AY, UK.

⁷ Atkinson Center for a Sustainable Future, Cornell University, Ithaca, NY 14850, USA.

⁸ Cornell Lab of Ornithology, Cornell University, Ithaca, NY 14850, USA.

⁹ Laboratorio de Ecología Molecular, Departamento de Ciencias Ecológicas II, Facultad de ciencias, Universidad de Chile. Las Encinas #3770, Ñuñoa, Santiago, Chile.

¹⁰ Pontificia Universidad Católica de Chile, Facultad de Agronomía e Ingeniería Forestal, Departamento de Ecosistemas y Medio Ambiente, Vicuña Mackenna 4860, Macul, Santiago, Chile.

¹¹ Centre d'Etudes Biologiques de Chizé (CEBC), UMR 7372 du CNRS-La Rochelle Université 79360 Villiers-en-Bois, France.

¹² Royal Society for the Protection of Birds, The Lodge, Sandy, Bedfordshire, SG19 2DL, UK.

¹³ World Land Trust, Blyth House, Halesworth, Suffolk, IP19 8AB, UK.

¹⁴ Department of Ecology, Environment and Evolution, La Trobe University, Melbourne, Australia.

¹⁵ Global Penguin Society, University of Washington, Seattle, USA.

¹⁶ Department of Zoology, University of Oxford, Peter Medawar Building for Pathogen Research, South Parks Road, OX1 3SY, UK.

¹⁷ Department of Conservation, Murihiku District Office, Invercargill, New Zealand.

¹⁸ Biodiversity, Department of Conservation, Auckland, New Zealand.

¹⁹ Centre d'Ecologie Fonctionnelle et Evolutive, UMR 5175, Campus CNRS, 1919 Route de Mende, 34293 Montpellier Cedex 5, France.

²⁰ Division of Pathology and Laboratory Medicine, University of Western Australia, Crawley, Western Australia, 6009, Australia.

²¹ Institute for Marine and Antarctic Studies, University of Tasmania, Hobart, Tasmania, 7001, Australia.

²² Museum of New Zealand Te Papa Tongarewa, PO Box 467, Wellington 6140, New Zealand.

²³ Department of Biology, The Citadel, 171 Moultrie St, Charleston, SC, 29409, USA.

²⁴ Department of Oceanography and Coastal Sciences, Louisiana State University, 1239 Energy, Coast and Environment Building, Baton Rouge, LA 70803, USA.

²⁵ Justus Liebig Universität Giessen, Heinrich-Buff-Ring 26, 35392, Giessen, Germany.

²⁶ FitzPatrick Institute of African Ornithology, University of Cape Town, Rondebosch 7701, South Africa.

²⁷ National Institute of Water and Atmospheric Research Ltd., Private Bag 14901, Kilbirnie, Wellington 6241. New Zealand.

²⁸ Department of the Environment and Energy, Australian Antarctic Division, 203 Channel Highway, Kingston, TAS 7050, Australia.

ORCID numbers: 0000-0002-0197-286X (T.L. Cole), 0000-0002-8302-0366 (M-J. Frugone), 0000-0001-9469-9489 (Y. Cherel), 0000-0002-6456-7757 (A. Alexander), 0000-0002-1514-7916 (J.M. Waters).

^a Corresponding author

^b LD, ND, TH and AA contributed equally to this work

Abstract

Climate shifts are key drivers of ecosystem change. Despite the critical importance of Antarctica and the Southern Ocean for global climate, the extent of climate-driven ecological change in this region remains controversial. In particular, the biological effects of changing sea-ice conditions are poorly understood. We hypothesise that rapid postglacial reductions in sea-ice drove biological shifts across multiple widespread Southern Ocean species. We test for demographic shifts driven by climate events over recent millennia by analysing population genomic datasets spanning three penguin genera (*Eudyptes*, *Pygoscelis* and *Aptenodytes*). Demographic analyses for multiple species (macaroni/royal, eastern rockhopper, Adélie, gentoo, king and emperor) currently inhabiting southern coastlines affected by heavy sea-ice conditions during the Last Glacial Maximum (LGM) yielded genetic signatures of near-simultaneous population expansions associated with post-glacial warming. Populations of the ice-adapted emperor penguin are inferred to have expanded slightly earlier than those of species requiring ice-free terrain. These concerted high-latitude expansion events contrast with relatively stable/declining demographic histories inferred for four penguin species (northern rockhopper, western rockhopper, Fiordland crested and Snares crested) that apparently persisted throughout the LGM in ice-free habitats. Limited genetic structure detected in all ice-affected species across the vast Southern Ocean may reflect both rapid post-glacial colonisation of sub-Antarctic and Antarctic shores, in addition to recent genetic exchange among populations. Together, these analyses highlight dramatic, ecosystem-wide responses to past Southern Ocean climate change, and suggest potential for further shifts as warming continues.

Keywords

Sphenisciformes, Climate Change, Last Glacial Maximum, Refugia, Genomics.

Significance statement

We analyse population genomic datasets across three penguin genera to test for demographic shifts driven by historical climate events. Numerous species inhabiting coastlines affected by heavy sea-ice during the Last Glacial Maximum show genomic signatures of near-simultaneous population expansions associated with post-glacial warming, contrasting with stable/declining demographic histories inferred for four species occupying consistently ice-free habitats. Shallow population genomic structure detected within species distributed across the vast Southern Ocean likely provides further evidence for recent demographic shifts, and recent genetic exchange among populations. Our results demonstrate dramatic, ecosystem-wide responses to climate change, and highlight the potential for future biological shifts in the Southern Ocean as global warming continues.

Introduction

Climate change is substantially impacting the abundance and distribution of wildlife, with many species' ranges shifting poleward as a result of climate warming (1). Similar shifts occurred after the Last Glacial Maximum (LGM; 18,000 –25,000 years ago; [2-3]), as temperate refugial populations of many species expanded into high latitudes. While such range shifts may be readily achieved on continents (where terrestrial habitats are essentially continuous [4]), the challenges are more pronounced for isolated or fragmented populations that rely on long-distance dispersal (5-6). For instance, many high-latitude coastal and terrestrial ecosystems of the Southern Hemisphere are isolated by vast ocean gaps (Fig. 1). Southern Ocean circumpolar fronts (including the Subtropical Front and the Antarctic Polar Front) may present additional physical and thermal barriers to southward range expansion of isolated southern coastal populations (10-11).

Understanding past shifts in species distributions is crucial for forecasting responses to contemporary and future climate change. Currently, there is considerable uncertainty surrounding the extent to which high-latitude wildlife populations might have persisted in the Southern Ocean throughout the LGM, versus the extent of post-LGM expansion (6-7, 12). Recent genetic data, however, hint at major ecosystem-wide change following reductions in southern winter sea-ice (7, 13-14). Importantly, past expansions can be reconstructed via genetic analysis of modern populations (2, 15). While several studies of Southern Ocean species have detected limited population genetic structure, consistent with recent demographic shifts and/or gene flow (9, 13-14, 16-19), a comprehensive genome-wide assessment of Southern Ocean wildlife is lacking. Moreover, as responses to climate change can potentially vary among species (14, 20-21), distinguishing between concerted (multi-species) versus idiosyncratic (single species) shifts may be crucial to forecasting responses to future climate change (22).

Penguins (Sphenisciformes) are iconic marine birds that inhabit all major southern landmasses, with their greatest species diversity in Antarctica and the sub-Antarctic (Fig. 1; SI Appendix, Fig. S1). Although most penguins are natally philopatric (23), some can disperse vast distances traversing major Southern Ocean fronts (24-25), and represent important components of both coastal and marine ecosystems (26). Here we analyse several thousand single nucleotide polymorphisms (SNPs) across 11 Antarctic, sub-Antarctic and temperate penguin species to test for concerted responses to climate change. We detect genomic signatures of population expansion in multiple species currently distributed largely within the LGM sea-ice zone, consistent with concerted re-colonisation of Antarctic and sub-Antarctic coasts during post-LGM warming. In contrast, demographic histories inferred for four temperate penguin species are relatively stable/declining. Our results suggest consistent

population dynamics across a species-rich high-latitude assemblage in response to postglacial ice reduction, and demonstrate the potential for rapid change to Southern Ocean ecosystems under future warming.

Results

Demographic reconstructions of effective population sizes (N_e) for 11 penguin species using CubSFS (28), SNAPP (29), Tajima's D (30) and Multi-dice (31) were based on 3,000-13,000 SNPs per species (SI Appendix, Tables S1-S3). Macaroni and royal [*Eudyptes chrysolophus chrysolophus*/*E. c. schlegeli*] penguins were considered a single species based on structure/ F_{ST} analyses (see also (19)), whereas Snares-crested [*E. robustus*] and the northern rockhopper [*E. moseleyi*] penguin were excluded from some analyses due to their small sample sizes (Fig. 1, SI Appendix, Tables S4-S5). These analyses revealed comparable postglacial N_e expansions for six southern species (macaroni/royal, eastern rockhopper [*E. filholi*], Adélie [*Pygoscelis adeliae*], gentoo [*P. papua*], king [*Aptenodytes patagonicus*] and emperor [*A. forsteri*] penguins) (Fig. 2, Fig. 3a; SI Appendix, Table S1, Figs. S2-S3), with the emperor penguin expanding slightly earlier. Additionally, two of three demographic analyses supported recent expansion in a seventh species (chinstrap [*Pygoscelis antarctica*]) (Fig. 3a). Notably, these seven species all predominantly occur south of the LGM sea-ice limit (Fig. 1; see [6-8, 23]). By contrast, four species inferred to have relatively stable/declining recent demographic histories (Fig. 3a, Figs. S2-S3) are all predominantly found north of the LGM sea-ice zone (Figs. 1-2): the northern rockhopper (*Eudyptes moseleyi*; Gough and Amsterdam Islands), western rockhopper (*E. chrysocome*; predominantly the Falkland Islands and southern South America), Fiordland-crested (*E. pachyrhynchus*, southern New Zealand) and Snares-crested (*E. robustus*, The Snares and Western Chain) penguins.

The expansion timeframes inferred for most southern lineages (20,000 – 15,000 years ago) correspond to a period of rapid post-LGM warming (27) (Fig. 2a; SI Appendix, Table S1). These reconstructions suggest populations of the ice-adapted emperor penguin expanded earlier than those of most other southern penguin lineages which require ice-free terrain (see also [16, 32]). The magnitude of inferred postglacial N_e expansions is on average a 2.7-fold increase (ranging from 1.19 – 4.4 fold increase) (Fig. 2a; SI Appendix, Table S1). We detected some variation in the outcomes of different demographic analyses for particular species, perhaps a reflection of varying sensitivity of different model-based approaches and/or biological signal. For example, the CubSFS analysis contrasted with other approaches in suggesting chinstrap penguin populations expanded prior to the LGM, and declined following the LGM. Overall, however, there is broad support for ‘stable/declining’ demographic trajectories for species inhabiting LGM ice-free regions, versus predominantly ‘expanding’ trajectories for LGM ice-affected species (Fig. 3a).

We used Multi-dice to test for synchronous versus asynchronous expansions across the seven ‘expanded’ species identified based on our demographic analyses (Fig. 3a). To this end, we modelled a single expansion event within the last 50,000 years in which up to seven species co-expanded. The synchronous expansion event was inferred to have occurred 20,779 – 24,804 years ago, depending on the summary statistics chosen (Fig. 3b; SI Appendix, Table S3). While only two or three of these southern species were inferred to have expanded simultaneously (SI Appendix, Table S3), minor differences between inferred expansion timings (Fig. 2a) likely hindered the ability for Multi-dice to detect a single expansion event corresponding to all expanding species.

Tests for intraspecific genomic divergence across the ranges of individual species (including previous analyses of *Pygoscelis* and *Aptenodytes* species; see [13, 14, 33-34]) consistently revealed shallow genetic structure within species (Fig 1; SI Appendix, Figs. S4-S7; SI Appendix, Tables S6-S7). In all cases apart from gentoo penguins, we found that panmixia ($K=1$) was supported, but that using location priors found evidence for additional fine-scale structure, as previously reported [9, 13, 14, 33-34]. Such patterns are consistent with post-LGM demographic and biogeographic expansions (for southern LGM sea-ice species) and recent genetic exchange among populations. Specifically, F_{st} , PCoA, Structure, DAPC, SNAPP and phylogenetic analyses for *Eudyptes*, *Pygoscelis* and *Aptenodytes* all revealed relatively shallow within-species genomic structure among southern populations (Fig 1; SI Appendix, Figs. S4-S7; SI Appendix, Tables S6-S7; [11, 16, 34-35]). In contrast to the recent genetic exchange inferred within most species, and between macaroni and royal penguins (18-19, 35), these analyses detected little or no admixture among species (Figs. S4-S7; SI Appendix).

Discussion

Our study detected broadly consistent genome-wide signatures of post-LGM expansion across penguin species that currently breed south of the LGM sea-ice zone (Fig. 3a). By contrast, four species currently breeding north of the LGM sea-ice zone exhibited genetic signatures of relatively stable/declining demographies (Fig. 3a). Although estimates of precise LGM breeding ranges for penguins remain elusive (but see [36]), our findings are consistent with the hypothesis of (6) that, during the LGM, many Southern Ocean species retreated to ice-free refugia (e.g. Gough, Amsterdam, Falklands islands, southern South America, and New Zealand's southern islands (Fig. 2b; see [6-7]). Indeed, several recent studies have suggested that post-LGM reductions in sea-ice were accompanied by rapid re-

colonisation of high-latitude shores (7, 11, 14) (Fig. 2c). Recent demographic studies of penguins (Adélie, emperor and king) (16-17, 32) and the southern elephant seal (37), for example, have inferred rapid postglacial recolonisation events. By contrast, recent snow-petrel analyses provide only limited evidence for such postglacial shifts (12). Choice of mutation rate, and possibly time-dependency issues might play some part in these apparently conflicting patterns among taxa. Some contrasting responses among species may also stem from interspecific ecological differences (e.g. variation in feeding ecology, philopatry, habitat preferences). Shifting oceanographic and coastal environmental features associated with postglacial warming may also have impacted local species.

While most LGM coasts are now inundated (see Fig. 2b), some potential LGM refugia may be suggested on the basis of current distributions (e.g. eastern rockhopper penguin likely expanded south from the Auckland, Campbell and Antipodes islands; [Fig. 2c]). Previous studies have concluded that the Southern Ocean's circumpolar fronts can represent important barriers to dispersal for many marine species (10, 11), including penguins (9, 38). However, several penguin species can clearly traverse such boundaries (24-25), and this exceptional dispersal ability may help to explain their apparently rapid biogeographic shifts in response to changing climate (see also [37]).

While CubSFS suggested the chinstrap penguin may have declined following the LGM, Tajima's D and SNAPP supported population expansion for this species, comparable to results for other southern species (Fig. 3a). This anomaly may perhaps reflect issues with the mutation rate and/or generation time used, or may indicate an idiosyncratic ecological response for this southern species (e.g. variation in feeding ecology, philopatry, habitat preferences, sensitivity to oceanographic fronts). Based on evidence from combined

demographic analyses (Fig. 3a), the suggestion that chinstrap penguins have declined since the LGM should be treated with some caution.

A consistent finding of our study is the lack of *major* genome-wide differentiation across the ranges of most penguin species, including several species showing circumpolar near-homogeneity (16-17) (i.e. $K = 1$; $F_{ST} < 0.02$; Fig. 1 and Table S6). These relatively shallow F_{ST} values contrast with more substantial structure, and evidence for multiple Southern Ocean refugia, in white chinned petrels ($K = 3$; $F_{ST} > 0.10$ (39)). While biallelic markers such as the SNPs analysed here are theoretically capable of yielding F_{ST} as high as 1 (i.e. fixed differences at all loci), we note that the upper range of this parameter can be limited by allele frequency distribution (40), and thus these values should be treated with some caution. While use of location priors at higher values of K reveals additional, fine-scale population differentiation (Fig. 1 and Fig. S4), see also (9, 33-34), such structure can potentially evolve rapidly (e.g. 41). Interestingly, the relatively shallow differentiation observed within and among some colonies (e.g. emperor (9, 34)) may also provide additional evidence of recent or ongoing gene flow and admixture, sometimes over vast distances (Fig. 1). Subtle population differentiation detectable with location priors might reflect the influence of contemporary oceanographic fronts and/or changes in local sea-ice conditions, as previously suggested by (9, 13, 17-18), and may have considerable relevance over ecological timeframes (e.g. conservation management; studies of migration).

Understanding how biota responded to past climate change is essential for predicting species distributions and population sizes under future climate projections, and for developing appropriate conservation management strategies (13, 42). As global temperatures continue to increase, mid-latitude biota will continue to shift towards the poles (11) or alternatively may

face extinction (6, 11). Many penguin populations are currently declining, or are predicted to decline as warming continues (43-45). Some of the northernmost colonies of Adélie and emperor penguins have already disappeared (43, 46), and in the case of emperor penguins, these changes have been linked directly to reductions in sea-ice (47). By contrast, populations of gentoo penguin are apparently expanding their ranges southward as the climate warms (48). Our study broadly demonstrates the demographic sensitivity of Southern Ocean wildlife to the effects of past climate change (49), highlighting the potential for future shifts under anthropogenic climate change.

Materials and Methods

DArT-Seq™ library preparation and filtering: DNA was extracted from 428 *Eudyptes* penguin samples spanning six species (Fig. 1; SI Appendix, Fig. S1; SI Appendix, Table S4; macaroni/royal penguins were combined; see [18-19]) using a modified Qiagen DNeasy Blood and Tissue kit. Library preparation and SNP discovery was performed on the highest quality DNA extracts using Diversity Arrays Technology Pty Ltd (DArT-seq™) in Canberra, Australia (50). Each sample was processed following (51), and was sequenced across three lanes on an Illumina Hiseq 2500. Sequences were processed using in-house proprietary DArT analytical pipelines. We used DartR v1.1.6 (52) in R v.3.5.1 (R Core Team, 2018) to filter the DArT-seq™ data for ten separate *Eudyptes* datasets (based on previous systematic discussions [18, 35], SI Appendix, Table S5). For these *Eudyptes* datasets, we filtered on reproducibility ($t=1$), and filtered out monomorphic loci, loci with call rates $<0.95\%$, all individuals with call rates $<0.90\%$, all loci with trimmed sequence tags, and all loci that departed from Hardy Weinberg Equilibrium in any colony ($P = 0.05$ following Bonferroni correction). We also obtained filtered RAD-seq datasets from an additional five penguin species generated and examined by (9, 33-34), comprising Adélie (*Pygoscelis*

adeliae; $n=87$), gentoo (*P. papua*; $n=36$), chinstrap (*P. antarctica*; $n=44$), king (*Aptenodytes*
patagonicus; $n=64$) and emperor (*A. forsteri*; $n=110$) penguins (SI Appendix, Table S8). See
SI Appendix for details.

Phylogenomic analysis and population structure: To clarify the evolutionary relationships
among our *Eudyptes* samples newly sequenced in this study, we created a maximum
likelihood phylogeny using RAxML-HPC v.8.2.1 (53) (SI Appendix, Fig. S5). We undertook
similar population structure analyses for *Eudyptes* as previously implemented for *Pygoscelis*
and *Aptenodytes* in (9), as follows: we calculated population summary statistics, including the
number of private alleles, observed and expected heterozygosity, the inbreeding coefficient,
and global and pairwise F_{ST} (Fig. 1, Tables S5-S7). Genetic clusters were visualised using
three methods: principal coordinates analyses (PCoA) using adegenet (54) (SI Appendix, Fig.
S4); the Evanno method (55) in Structure v.2.3.4 (56), to estimate the most likely K (Fig. 1;
SI Appendix, Fig. S4); and discriminant analysis of principal components (DAPC) using
adegenet (SI Appendix, Fig. S4). We used the SNAPP tree set analyser in BEAST v.2.4.7
(30, 57) to investigate gene flow between closely related *Eudyptes* species (SI Appendix, Fig.
S6), based on our results and systematic discussions of (18, 35). While SNAPP analyses have
been previously generated for the emperor, king and gentoo datasets (9, 33-34), we also
undertook SNAPP analyses for the chinstrap and Adélie penguin datasets obtained from (9)
(SI Appendix, Fig. S7). See SI Appendix for details.

Testing for demographic expansions: We reconstructed population histories for 11 *Eudyptes*,
Pygoscelis and *Aptenodytes* species over the last 1,000,000 years, by estimating the time and
magnitude of demographic changes using four different approaches (northern rockhopper and
Snares crested penguin were excluded from some analyses due to low sample size).

Specifically, we reconstructed the demographic histories using CubSFS (Fig. 2A; SI Appendix, Figs. S2-S3; SI Appendix, Table S1); obtained Tajima's D (SI Appendix, Table S2); identified the change in theta values as inferred by our previous SNAPP analyses (SI Appendix, Figs. S8-S9); and tested for synchronous expansion using Multi-dice (Fig. 3b; SI Appendix, Table S3). As the *Eudyptes* and *Pygoscelis/Aptenodytes* datasets were obtained using different pipelines (DART-seqTM versus RAD-seq), we applied further stringent filtering to ensure consistency between the datasets (SI Appendix). While previous studies have reported shallow population genetic structure within most *Pygoscelis* and *Aptenodytes* species (9, 13, 16-17, 33-34) (Fig. 1), given the relatively shallow F_{ST} values involved, and reported $K = 1$ ([9, 33-34]), we consider this fine-scale structure likely to have evolved recently, and broadly consistent with a scenario of high gene flow, suitable for combined demographic analysis (with the exception of the gentoo penguin (9)). To account for the deeper genetic structure observed in gentoo populations (e.g. four distinct lineages [9]; Fig. 1), we limited most subsequent analyses of this species to one lineage (Fig. 1; see [9]). For each *Pygoscelis*, *Eudyptes* and *Aptenodytes* vcf file, we projected the folded allele frequency spectrum down to increase the number of segregating sites using EasySFS. We then adjusted the number of monomorphic sites in our allele frequency spectrum to reflect the total number of monomorphic loci within each species following down projection. For all analyses, we assumed a generation time of 8 – 14 years (from [42]). For all models, we used a mutation rate of 2.6×10^{-7} per locus per generation (14, 16).

Acknowledgements

We thank C. Bazjak, A-S. Coquel, N. Dehnhard, M. Fawcett, H. Irvine, K. Morrison and M. Nicolaus for sample collection, T. King, C. Mitchell and K. Trought for laboratory assistance, V. Chhatre, A. Georges, B. Gruber, F. Pinamartins, B. Roberts, A. Savary, J. Wood and A.

330 Xue for bioinformatics assistance and L. Beheregaray, C. Fraser, A. Kilian, M. Knapp, A.
 331 Ruzzante, A. Santure, P. Scofield, P. Sunnucks, J. Wilmshurst and J. Wood for discussions.
 332 The research was approved by the Otago University Animal Ethics Committee (AEC)
 333 61/2016, Oxford University Local AEC, University of Western Australia AEC, Woods Hole
 334 Oceanographic Institution Animal Care and Use Committee, IPEV ethics committee, Otago
 335 University Ngāi Tahu Research Consultation Committee and The Zoological Society of
 336 London. Work was carried out under NZ Department of Conservation Permits (OT-25557-
 337 DOA, IACUC-18958.00, 32202-FAU, 35682-FAU, 37312-LND, 50437-DOA, 50436-FAU,
 338 50464-DOA, and 38882-RES), NZ Ministry of Primary Industries (2015056535,
 339 2016060908, 2017064905), a Permit to Possess Threatened Fauna for Scientific Purposes No.
 340 TFA 15086, DPIWE Permit to Take Wildlife for Scientific Purposes No. FA05246, Tristan
 341 Da Cunha Conservation Department, South African Department of Environmental Affairs,
 342 Government of South Georgia and the South Sandwich Islands Restricted Activity Permits
 343 (GSGSSI RAP 2018/018, GSGSSI), US NSF Department of Polar Programs ACA permits
 344 (ACA 2016-011, ACA 2016-012), Falkland Islands Government (R05/2009, R014/2006) and
 345 UK Antarctic Permits. We thank N. Fowke and B. McKinlay for NZ permits. The research
 346 was supported by Manaaki Whenua Landcare Research, the University of Otago, Museum of
 347 NZ Te Papa Tongarewa, US National Science Foundation (OPP-012-8913), Quark
 348 Expeditions, Cheesemans Ecology Safaris, Golden Fleece Expeditions, New Island
 349 Conservation Trust, Deutsche Forschungsgemeinschaft, The Citadel Foundation, The Dalio
 350 Foundation, donations to Penguin Watch, the Institut Polaire Français Paul Emile Victor
 351 (IPEV, Programme N°109, P. Jouventin, H. Weimerskirch, and N°134, C.A. Bost), The
 352 Royal Society of NZ Hutton Fund, The Ornithological Society of NZ and an Alumni of
 353 Otago in America Award. TLC was supported by an Otago University Postgraduate Award.

354 **Author contributions**

TLC, TH, JMW and ND conceived and designed the study. TLC, LD, ND, AA, JLY and GVC analysed the data. TLC and SRF undertook laboratory work. TLC, TH, ND, JLY, GVC, YC, RC, UE, SRF, DH, PJ, TM, GM, CM, PN, MJP, PQ, PGR, AS, AJDT, DT and BW collected samples. TLC, TH, JMW, LD, ND and AA wrote the manuscript. All authors contributed to the manuscript.

Data availability

Raw fastq reads are available from the Short Read Archive (DOI:10.6084/m9.figshare.c.4475300). Additional DArT-Seq files, Structure and SNAPP input and output files, and the original and amended SFS are also available on FigShare (DOI:10.6084/m9.figshare.c.4475300).

References

1. Chen I-C, Hill JK, Ohlemuller R, Roy DB, Thomas CD. Rapid range shifts of species associated with high levels of climate warming. *Science*. **333**, 1024–1026 (2011).
2. Hewitt G. The genetic legacy of the Quaternary ice ages. *Nature*. **405**, 907–913 (2000).
3. Davis MB, Shaw RG. Range shifts and adaptive responses to Quaternary climate change. *Science*. **292**, 673–679 (2001).
4. Parmesan C, Yohe GA. globally coherent fingerprint of climate change impacts across natural systems. *Nature*. **421**, 37–42 (2003).
5. Trakhenbrot A, Nathan R, Perry G, Richardson DM. The importance of long-distance dispersal in biodiversity conservation. *Divers. Distrib.* **11**, 173 (2005).
6. Fraser CI, Nikula R, Ruzzante DE, Waters JM. Poleward bound: biological impacts of Southern Hemisphere glaciation. *Trends Ecol. Evol.* **27**, 462–471 (2011).
7. Fraser CI, Nikula R, Spencer HG, Waters JM. Kelp genes reveal effects of subantarctic sea ice during the Last Glacial Maximum. *P. Nat. Acad. Sci. USA*. **106**, 3249–3253 (2009).
8. Gersonde R, Crosta X, Abelmann A, Armand L. Sea-surface temperature and sea ice distribution of the Southern Ocean at the EPILOG Last Glacial Maximum – a circum-Antarctic view based on siliceous microfossil records. *Quat. Sci. Rev.* **24**, 869–896 (2005).
9. Clucas GV, et al. Comparative population genomics reveals key barriers to dispersal in Southern Ocean penguins. *Mol. Ecol.* **27**, 4680–4697 (2018).

10. Munro KJ, Burg TM. A review of historical and contemporary processes affecting population genetic structure of Southern Ocean seabirds. *Emu*. **117**, 4–18 (2017).
11. Fraser CI, et al. Antarctica’s ecological isolation will be broken by storm-driven dispersal and warming. *Nat. Clim. Change*. **8**, 604–708 (2018).
12. Carrea C, et al. High vagility facilitates population persistence and expansion prior to the Last Glacial Maximum in an Antarctic top predator: the snow petrel (*Pagodroma nivea*). *J. Biogeog.* **46**, 442–453 (2019).
13. Clucas GV, et al. A reversal of fortunes: climate change ‘winners’ and ‘losers’ in Antarctic Peninsula penguins. *Sci. Rep.* **4**, 5024 (2014).
14. Trucchi E, et al. King penguin demography since the last glaciation inferred from genome-wide data. *Proc. R. Soc. Lond. B.* **281**, 20140528 (2014).
15. Waters JM, Fraser CI, Hewitt GM. Founder takes all: density-dependent processes structure biodiversity. *Trends Ecol. Evol.* **28**, 78–85 (2013).
16. Cristofari R, et al. Full circumpolar migration ensures evolutionary unity in the emperor penguin. *Nat. Commun.* **7**, 11842 (2016).
17. Cristofari R, et al. Climate-driven range shifts of the king penguin in a fragmented ecosystem. *Nat. Clim. Change*. **8**, 245–251 (2018).
18. Frugone M-J, et al. Contrasting phylogeographic pattern among *Eudyptes* penguins around the Southern Ocean. *Sci. Rep.* **8**, 17481 (2018).
19. Frugone N-J, et al. More than the eye can see: Genomic insights into the drivers of genetic differentiation in Royal/Macaroni penguins across the Southern Ocean. *Mol. Phylogenet. Evol.* **139**, 106563 (2019).
20. Maggs CA, et al. Evaluating signatures of glacial refugia for North Atlantic benthic marine taxa. *Ecology*. **89**, S108–S122 (2008).
21. Stewart JR, Lister AM, Barnes I, Dalén L. Refugia revisited: individualistic responses of species in space and time. *Proc. R. Soc. Lond. B: Biol. Sci.* **277**, 661–671 (2010).
22. Walther G-R, et al. Ecological responses to recent climate change. *Nature*. **416**, 389 (2002).
23. Borboroglu PG, Boersma PD (eds). *Biology and Conservation of the World’s penguins*, University of Washington Press, Seattle U.S.A. (2013).
24. Thiébot JB, Cherel Y, Trathan PN, Bost CA. Coexistence of oceanic predators on wintering areas explained by population-scale foraging segregation in space or time. *Ecology*. **93**, 122–130 (2012).
25. Thiébot JB, Cherel Y, Trathan PN, Bost CA. Inter-population segregation in the wintering areas of macaroni penguins. *Mar. Ecol. Prog. Ser.* **421**, 279–290 (2011).

26. Woehler EJ, et al. A statistical assessment of the status and trends of Antarctic and subantarctic seabirds. Scientific Committee on Antarctic Research, Cambridge, UK. (2011).
27. Jouzel J, et al. Orbital and millennial Antarctic climate variability over the past 800,000 years. *Science*. **317**, 793–796 (2007).
28. Waltoft BL, Hobolth A. Non-parametric estimation of population size changes from the site frequency spectrum. *Stat. Appl. Genet. Mo. Biol.* **17**, 3 (2018).
29. Bouckaert R, Heled J. DensiTree2: seeing trees through the forest. *BioRxiv*. 012401. (2014).
30. Gutenkunst RN, Hernandez RD, Williamson SH, Bustamante CD. Inferring the Joint Demographic History of Multiple Populations from Multidimensional SNP Frequency Data. *PLOS Genet.* **5**, e1000695 (2009).
31. Xue AT, Hickerson MJ. Multi-dice: r package for comparative population genomic inference under hierarchical co-demographic models of independent single-population size changes. *Mol. Ecol. Res.* **17**, e212–e224 (2017).
32. Li C, et al. Two Antarctic penguin genomes reveal insights into their evolutionary history and molecular changes related to the Antarctic environment. *GigaScience*. **3**, 27 (2014).
33. Clucas GV, et al. Dispersal in the sub-Antarctic: king penguins show remarkably little population genetic differentiation across their range. *BMC Evol. Biol.* **16**, 211 (2016).
34. Younger J, et al. The challenges of detecting subtle population structure and its importance for the conservation of Emperor penguins. *Mol. Ecol.* **26**, 3883–3897 (2017).
35. Cole TL, et al. Mitogenomes uncover extinct penguin taxa and reveal island formation as a key driver of speciation. *Mol. Biol. Evol.* **36**, 784–797 (2019).
36. Emslie SD, McKenzie A & Patterson WP. The rise and fall of an ancient Adélie penguin ‘supercolony’ at Cape Adare, Antarctica. *Roy. Soc. Open Sci.* **54**, 172032 (2018).
37. de Bruyn M, et al. Rapid response of a marine mammal species to Holocene climate and habitat change. *Plos. Gen.* **5**, e1000554 (2009).
38. de Dinechin M, Ottval R, Quillfeldt P, Jouventin P. Speciation chronology of rockhopper penguins inferred from molecular, geological and palaeoceanographic data. *J. Biogeog.* **36**, 693 – 702 (2009).
39. Rexer-Huber, et al. Genomics detects population structure within and between ocean basins in a circumpolar seabird: the white-chinned petrel. *Mol. Ecol.* (In Press).
40. Alcalá N & Rosenberg NA. Mathematical constraints on F_{ST} : biallelic markers in arbitrarily many populations. *Genetics*. **206**, 1581–1600 (2017).

41. Boessenkool S, Star B, Waters JM, Seddon PJ. Multilocus assignment analyses reveal multiple units and rare migration events in the recently expanded yellow-eyed penguin (*Megadyptes antipodes*). *Mol. Ecol.* **18**, 2390–2400 (2009).
42. Forcada J, Trathan PN. Penguin responses to climate change in the Southern Ocean. *Glob. Change Biol.* **15**, 1618–1630 (2009).
43. Forcada J, Trathan PN, Reid K, Murphy EJ, Croxall JP. Contrasting population changes in sympatric penguin species in association with climate warming. *Glob. Change Biol.* **12**, 411–423 (2006).
44. Robert-Coudert, et al. Happy feet in a hostile world? The future of penguins depends on proactive management of current and expected threats. *Front. Mar. Sci.* **6**, 248 (2019).
45. Boersma PD, et al. Applying science to pressing conservation needs for penguins. *Cons. Biol.* (In Press).
46. Barbraud C, Weimerskirch H. Emperor penguins and climate change. *Nature.* **411**, 183–186 (2001).
47. Fretwell PT & Trathan PN. Emperors on thin ice: three years of breeding failure at Halley Bay. *Antarct. Sci.* (In Press).
48. Lynch HJ, Naveen R, Trathan PN, Fagan WF. Spatially integrated assessment reveals widespread changes in penguin populations on the Antarctic Peninsula. *Ecology.* **93**, 1367–1377 (2012).
49. Younger JL, van den Hoff J, Wienecke B, Hindell M, Miller KJ. Contrasting responses to climate regime change by sympatric, ice-dependent predators. *BMC Evol. Biol.* **16**, 61 (2016).
50. Kilian A, et al. Diversity Arrays Technology: a generic genome profiling technology on open platforms. *Meth. Mol. Biol.* **888**, 67–89 (2012).
51. Sansaloni C, et al. Diversity Arrays Technology (DArT) and next generation sequencing combined: genome-wide, high-throughput, highly informative genotyping for molecular breeding of *Eucalyptus*. *BMC Proceed.* **5**, 54 (2011).
52. Gruber B, Unmack PJ, Berry OF, Georges A. DARTR: An R package to facilitate analysis of SNP data generated from reduced representation genome sequencing. *Mol. Ecol. Res.* **18**, 691–699 (2018).
53. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* **30**, 1312–1313 (2014).
54. Jombart T, Collins C. Analysing genome-wide SNP data using adegenet 2.0.0. (2015).
55. Evanno G, Regnaut S, Goudet J. Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* **14**, 2611–2620 (2005).

- 543 56. Pritchard JK, Stephens M, Donnelly P. Inference of population structure using multilocus
544 genotype data. *Genetics*. **155**, 945–959 (2000).
545
- 546 57. Bouckaert R, et al. BEAST 2: A software platform for Bayesian evolutionary analysis.
547 *PLOS Comput. Biol.* **10**, e1003537 (2014).

Figure Legends

Fig. 1. Sampling locations and genetic Structure plots for 11 penguin species (royal/macaroni are considered one species). The map (adapted from [6]) shows the Antarctic Circumpolar Current (ACC), the Subtropical Front (blue line), the Antarctic Polar Front (purple line), present summer (light blue shading) and winter sea-ice (mid blue shading), Last Glacial Maximum (LGM) winter sea-ice (dark blue shading) (see [6-7, 8]), LGM land extent (green) and glaciation during the LGM (white). Four species (indicated by squares) breed north of the LGM sea-ice limit, whereas seven species (indicated by circles) breed in southern regions affected by LGM sea-ice. The top Structure plot for each species (top two for gentoo) represents the most likely number of genetic clusters as determined via the Evanno method. The bottom Structure plot for each species shows a higher value of K to illustrate recently-evolved fine-scale genetic structure that can only be detected using location priors (Loc), as demonstrated by (9). Structure plots for Adélie, emperor, gentoo, king and chinstrap penguins are adapted from (9). With the exception of the gentoo penguin, all analyses demonstrated a most likely K of 1, with relatively shallow F_{ST} values (global F_{ST} is shown beside each species) (see [9]). Numerical codes for sampling locations (details in SI Appendix, Fig. S1) are indicated on the map and underneath structure plots. Sampling localities: Falkland Islands (FAL, PEB, NEW); South Shetland Islands (SSH); Elephant Island (ELE); South Orkney Islands (SOR); South Georgia (SGE); South Sandwich Islands (SSI); Bouvet (BOU); Gough Island and Tristan da Cunha (GOU); Marion Island (MAR), Prince Edward Islands (PEI); Crozet (CRZ); Kerguelen (KER); Amsterdam Island (AMS); Macquarie Island (MAC); Campbell Island (CAM); Auckland Islands (AUC); Antipodes Islands (ANT); The Snares (SNA), Western Chain (WES); Codfish Island (COD), Milford Sound (MIL), Jackson Head (JAC); Peterman Island (PET); Orne Harbour (ORN); Jougla Point (JOU); George's Point (GEO); Brown Bluff (BRO); Gould Bay (GOB); Halley Bay (HAL); Fold Island (FOL);

573 Béchervaise Island (BÉC); Auster (AUS); Welch Island (WEL); Amanda Bay (AMA);
574 Blakeney Point (BLA); Point Géologie (POI); Pétrels island (PÉT); Cape Roget (ROG);
575 Cape Washington (WAS). The asterix on Marion Island indicates the “white-faced”
576 phenotype of macaroni/royal penguin. Coloured symbols (squares/circles) are consistent with
577 Figs. 2 - 3.

578

579 **Fig. 2.** Population expansions/contractions of penguin species in relation to the LGM.
580 Species breeding south of the LGM sea-ice limit are represented by circles in A, B and C, and
581 species breeding north of the LGM sea-ice limit are represented by squares in A, B and C. A)
582 CubSFS demographic reconstructions for 10 penguin species (Snares crested penguin is
583 excluded due to low sample size). 95% confidence intervals are given by solid colour
584 intervals. Median for bootstrap replicates is given by the dotted line, and the solid line gives
585 the demographic reconstruction for the amended SFS. A 50 thousand year record of Antarctic
586 temperature change (grey line in each plot) as estimated from the EPICA Dome C Ice Core
587 [27] is shown in each plot. The grey bar in each plot shows the LGM. B) shows the winter
588 sea-ice and sea-level during the LGM, with putative refugia shown (orange ellipses for sub-
589 Antarctic penguins; grey points outlined in opaque white for all Antarctic penguins except the
590 emperor penguin). Arrows indicate likely glacial retractions of southern species in response
591 to LGM sea-ice (white arrows indicate retractions of Antarctic penguins to the fringes of the
592 summer sea-ice during the LGM [except the emperor penguin]; orange arrows indicate
593 retraction of sub-Antarctic penguins to refugial islands north of LGM sea-ice). The emperor
594 penguin presumably bred on the fringes of the summer sea-ice during the LGM (indicated by
595 pink points). Site names in black indicate possible refugia regions for sub-Antarctic penguins,
596 while site names in white indicate possible refugia regions for Antarctic penguins. C) shows
597 the present sea level and winter sea-ice extent, with possible post-LGM routes of

recolonization back to Antarctic and southern island habitats (white arrows for penguins breeding in Antarctica [except the emperor penguin]; yellow arrows for penguins breeding on southern islands). Regions where penguins likely persisted are shown with orange ellipses. The emperor penguin breeds on the fringes of the summer sea-ice, which is marked with pink points. Site names in black indicate where each penguin species currently breeds, while sites names marked in grey indicate locations where penguins may have bred during the LGM (as shown in B)). Note, these LGM breeding ranges in both B and C are uncertain. The maps have been adapted from (6). As the Snares crested penguin was included in other demographic analyses (see Fig. 3), the species is shown in both B and C. Coloured symbols (squares/circles) are consistent with Figs. 1 and 3.

Fig. 3. Summary of demographic results for 11 penguin species. A) shows the combined results of CubSFS, Tajima's D and SNAPP theta values. Species are broadly classified as 'expanding' (red: macaroni/royal, eastern rockhopper, Adélie, gentoo, chinstrap, king and emperor penguin [all south of the LGM sea-ice, represented by circles]) or 'declining/stable' (blue: northern rockhopper, western rockhopper, Fiordland crested, Snares crested penguin [all north of the LGM sea-ice, represented by squares]) on the basis of a majority of these analytical outputs. 'NA' indicates when a species was excluded from an analysis due to limited sample size. All analyses specifically address post-LGM demographic change, with the exception of Tajima's D which may also be influenced by earlier demographic events. B) Multi-dice results, suggesting a LGM expansion, with the mean of the co-expansion time parameter inferred at 24,065 years (mode: 20,778; median: 24,065). Coloured symbols (squares/circles) are consistent with Figs. 1 - 2.